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Application of solid-phase antibodies to radioimmunoassay

Evaluation of two polymeric microparticles, Dynospheres * and nylon, activated by carbonyldiimidazole or tresyl chloride

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Two types of polymeric microparticle, Dynospheres and reprecipitated acid-hydrolysed nylon 6/6, and two methods of activating these particles with either tresyl chloride or carbonyldiimidazole (CDI) prior to covalent linkage of antibodies were investigated with a view towards their respective adoption for the preparation of general solid-phase reagents for immunoassay applications. Activation of each particle and coupling of antibodies was rapid irrespective of the activator. CDI proved to be the activator of choice since it was cheap, less hazardous, more efficient and less pH dependent than tresyl chloride.

Both types of microparticle remain buoyant during the RIA incubation periods and form stable pellets after centrifugation. In second antibody applications immobilisation of the first antibody occurs with a short incubation period of 30 min. Nylon microparticles have a higher antibody-coupling capacity and are the particles of choice in both first and second antibody applications. However, the nylon microparticles possess marginally higher non-specific binding characteristics.

Key words: Dynospheres; Nylon; Carbonyldiimidazole; Tresyl chloride; Solid-phase antibodies

Introduction

The analytical principle of limited reagent immunoassay techniques is dependent upon two major factors; firstly, the presence of an analyte-specific antibody at limiting saturable concentration and secondly, estimations of the proportions of analyte partitioned between antibody-bound and free fractions. The latter is usually monitored by the incorporation of a trace of labelled analyte, usually but not exclusively radioactivity, and an unknown analyte quantitated by comparing its distribution with that of a set of analyte standards (Ekins, 1974).

The majority of immunoassays in clinical chemistry laboratories, with the exception of the 'homogeneous' non-separation assays, use physical separation techniques, e.g., electrophoresis, gel permeation, adsorption and precipitation to differentiate between bound and free fractions, as reviewed by Ratcliffe (1974, 1983). Currently, the

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* Dynospheres is a registered trademark of Dyno Industries A-S, Lillestrøm, Norway.

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Abbreviations: CDI, 1,1'-carbonyldiimidazole; T_4, thyroxine; T_3, triiodothyronine; LH, luteinizing hormone; RIA, radioimmunoassay; IgG, immunoglobulin G; DASG, donkey anti-sheep/goat; DAR, donkey anti-rabbit; FSH, follicle-stimulating hormone; GH, growth hormone; tresyl chloride, trifluoroethane sulphonyl chloride; BSA, bovine serum albumin; ANS, 8-anilinonaphthalene 1-sulphonate; EPPS, N-(2-hydroxyethyl)piperazine-N'-3-propanesulphonic acid.

EXHIBIT "A"

most efficient and practical separation systems involve either the covalent linkage of the primary analyte-specific antibody to particulate solid-phases (e.g., cellulose or dextran) (Wide and Porath, 1966) or the use of 'second' or 'double' anti-species antibodies designed to precipitate the primary antibody under optimised conditions (Hales and Randle, 1963). The latter can also be covalently linked to solid-phase particles, usually particles of high capacity, e.g., Sepharose (Den Hollander and Schuurs, 1971). In either case, a simple post-reaction centrifugation step separates antibody-bound and free fractions.

Solid-phase systems are increasing in popularity since virtually complete separation of the fractions can be achieved, particularly if washing systems are included. This yields assays of improved precision and bias, free from misclassification errors and consequently more rugged than their liquid-phase counterparts. Similarly, solid-phase assays are in general quicker to perform, not requiring the extra time to form insoluble precipitates, although conventional liquid-phase double antibody systems can be accelerated by the addition of polyethylene glycol (Edwards, 1983).

Ideally, for the solid-phase approach to be acceptable in routine clinical chemistry laboratories, several criteria must be fulfilled. Firstly, the solid-phase material chosen should be readily available and cheap, with a particle size small enough for it to remain buoyant during incubation but large enough to form a stable pellet after centrifugation. Secondly, the material should have surface groups commensurate with uncomplicated, non-toxic activation and coupling chemistries, with sufficient capacity for both primary and second antibody solid-phase applications. Finally, the preparation and subsequent application of the adopted solid-phase system should not be more tedious in practice than the system which it intends to replace.

In an attempt to fulfil these criteria two solid-phase microparticulate polymers, Dynospheres and nylon, and two activation chemistries involving either tresyl chloride or carbonyldiimidazole (CDI) were studied in detail with a view to providing solid-phase reagents suitable for use in a routine clinical chemistry laboratory.

Materials

The following materials were purchased from commercial sources: 1,1'-carbonyldiimidazole, thyroxine and triiodothyronine (sodium salts) (Sigma Chemical Co., Dorset, U.K.); 2,2,2-trifluoroethane sulphonyl chloride (Fluka, Fluorochem, Glossop, U.K.); Dynospheres XP4101 (Dyno Industries A-S, Lillestrøm, Norway); nylon 6/6 (poly(hexamethylene adipamide)) (Aldrich Chemical Co., Gillingham, U.K.); concentrated hydrochloric acid, pyridine, acetone, ethanolamine, buffer salts and additional reagents (Analar grade) (BDH, Poole, Dorset, U.K.).

Radioiodinated thyroxine (T_4, L-[^{125}I], NEX111, specific activity approximately 150 μCi/μg) and triiodothyronine (T_3, L-3,5,3'-[^{125}I], NEX110H, specific activity approximately 1200 μCi/μg) were purchased from New England Nuclear (Du Pont (U.K), Herts, U.K.). Radioiodinated luteinising hormone was made locally using the lactoperoxidase procedure (Karonen et al., 1975).

Sheep antisera to thyroxine (pool 066/7) and triiodothyronine (pool 083), together with donkey anti-sheep/goat and donkey anti-rabbit serum, were obtained from the Scottish Antibody Production Unit (Law Hospital, Carluke Lanarkshire, U.K.). Antiserum to luteinising hormone (WRB/F87-2-83) was a gift from Professor Butt (Birmingham and Midlands Hospital for Women, Birmingham, U.K.).

Methods

(1) Preparation of microparticulate nylon

Microparticulate nylon was prepared by a modification of the procedure described by Hendry and Herrmann (1980). Nylon 6/6 pellets were dissolved overnight (approximately 18 h) in hydrochloric acid, specific gravity 1.18 (40 ml/g nylon). The dissolved nylon was then recovered by dropwise addition of the acid solution to stirred distilled water (2 l/g nylon) from a glass pasteur pipette. Average particle size was estimated to be approximately 1 μm by microscopy. The particles were collected by centrifugation, followed by filtration on a glass microfibre filter (Whatman

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GF/A, Maidstone, U.K.) under reduced pressure. The particles were washed in acetone prior to activation.

(2) Preparation of IgG from antisera

Partially purified IgG was prepared from animal antisera by the *n*-octanoic acid fractionation technique of Steinbuch and Audran (1969). Essentially, 20 ml of antiserum was adjusted to pH 5 by dropwise addition of 0.1 M acetic acid, then 1.76 ml of *n*-octanoic acid was added slowly with vigorous stirring. After 30 min, the mixture was centrifuged and the IgG in solution removed. An improved recovery of IgG was achieved by washing the precipitate. The IgG solution was then extensively dialysed against 0.01 M sodium bicarbonate (pH 8) and concentrated to 50 mg/ml by ultrafiltration, before storage at −20°C. The protein concentration was then determined by the method of Schacterle and Pollack (1973).

(3) Activation of Dynospheres with tresyl chloride

Essentially, the solid-phase activation followed the procedure described by Nustad et al. (1982, 1984). Dynospheres were received from the manufacturers as a 10% w/v suspension. 10 ml (1 g) were transferred to a glass filtration apparatus (supplied by Millipore (U.K.), Harrow, Middlesex, U.K.) containing a 0.45 μm nylon filter (Anachem, Luton, U.K.), washed under reduced pressure with 50 ml acetone and allowed to air dry. The dried particles were subsequently placed into a conical glass test tube containing a triangular polytetrafluoroethylene-coated magnetic spinbar (Radleys, Sawbridgeworth, Herts, U.K.), together with 11.2 ml acetone, 5.5 ml pyridine and 3.3 ml tresyl chloride. This gave a final Dynosphere concentration of 50 mg/ml and a molar ratio tresyl chloride: pyridine of 1:2.3. The reaction was vigorously stirred for 15 min at ambient temperature and the activated Dynospheres recovered and washed with 50 ml distilled water by filtration.

(4) Activation of Dynaspheres and nylon with CDI

Dynospheres and nylon microparticles were activated with CDI by a modification of the procedure used for microcrystalline cellulose by Chapman and Ratcliffe (1982), and Chapman et al. (1983). 1 g of either polymer was washed with 50 ml acetone and air dried as previously described before suspending in 20 ml acetone to give a final particle concentration of 50 mg/ml. 0.5 g solid CDI, equivalent to a concentration slightly in excess of 3 mmol/g particles was quickly added and the reactants stirred vigorously for 15 min at ambient temperature. The activated particles were washed in 50 ml distilled water and recovered by filtration.

(5) Covalent linkage of antibodies to activated polymer

Covalent linkage of IgG isolated from DAR serum acted as a model system to determine the optimal coupling conditions for each of the activated microparticles. Various conditions, time, pH, buffer molarity and concentration of IgG, were studied, and the optimal situations determined by examining the behaviour of the DAR IgG-linked microparticles, as solid-phase second antibodies in an LH RIA.

In general, each of the activated microparticle preparations was sonicated for about 3 min to disperse aggregates and suspended in an appropriate coupling buffer containing the DAR IgG. IgG coupling occurred by end-over-end mixing at ambient temperature for a defined period of time. After coupling, the microparticles were washed in 1 M ethanolamine/HCl, pH 9.5 to block any remaining reactive groups and were recovered by filtration before testing in the RIA.

(6) Radioimmunoassays for evaluation of the activated microparticles

(a) Solid-phase primary antibodies

IgG fractions of sheep anti-T_4 and anti-T_3, prepared as described previously, were covalently linked to each of the activated microparticles to form solid-phase primary antibody reagents. Each solid-phase antibody was studied in a radioimmunoassay for either T_4 or T_3 as follows:

(i) Thyroxine RIA. A range of T_4 standard solutions (0–300 nmol/l) in charcoal-treated human serum (0.05 ml) (Stockhill, 1979) was

incubated with 0.1 ml ^{125}I-T_4 (NEX111; 150 μCi/μg; 400 pg/tube) and 0.2 ml of each solid-phase antibody at various concentrations in 0.1 M phosphate buffer, 0.154 M sodium chloride, 0.1% BSA, 1.25 g/l ANS, pH 7.4 for 1 h at ambient temperature without shaking. Post incubation, 1.0 ml of 0.9% sodium chloride was added, the tubes centrifuged for 15 min at 1500 × g and the supernatant (free) fraction aspirated. The solid-phase (bound) fraction was then counted on an automated gamma counter for 30 s.

(ii) Triiodothyronine RIA. 0.1 ml aliquots of T_3 standards (0–15 nmol/l) in charcoal treated serum were incubated with 0.1 ml ^{125}I-T_3 (NEX110H, 1200 μCi/μg; 60 pg/tube) and 0.2 ml of each solid-phase antibody at various concentrations in 0.1 M phosphate buffer, 0.154 M sodium chloride, pH 7.4, containing 0.1% BSA and 0.34 g/l ANS for 2 h at ambient temperature without shaking. 1.0 ml 0.9% sodium chloride was added before centrifugation, separation and counting for 30 s.

(b) Solid-phase second antibody

IgG fractions from DAR and DASG sera were covalently linked to each of the activated microparticles. These reagents were examined as solid-phase second antibodies in LH RIAs using either rabbit or sheep primary anti-LH as appropriate.

(i) Luteinising hormone RIA. 0.1 ml ^{125}I-LH (20000 cpm; specific activity 50–100 μCi/μg) was incubated overnight (16–18 h) with either 0.1 ml rabbit anti-LH serum (WRB/F87-2-83, 1:200000 initial dilution) or 0.1 ml sheep anti-LH (SAPU S116, 1:500000 initial dilution) together with a further 0.1 ml assay diluent (0.05 M phosphate buffer, 0.05% BSA pH 7.4). Separation followed the addition of 0.1 ml of solid-phase DAR IgG or DASG IgG as appropriate. Various masses of solid-phase were studied both with and without shaking, usually with 30 min incubation. Again, 1.0 ml 0.9% sodium chloride containing either 0.1% Triton X-100 or 0.1% Brij-35 for nylon and 0.1% Tween 20 for Dynospheres was added before centrifugation (15 min, 1500 × g). The solid-phase (bound) fraction was counted for 30 s, and the efficiency of the solid-phase second antibodies assessed by the maximal degree of binding of ^{125}I-LH in each case.

Results

(1) Optimal activation of the microparticles

To determine the optimal concentration of activator, Dynospheres (50 mg) were reacted with either tresyl chloride or CDI at concentrations ranging from 3.7 to 36.7 mmol/g and 0.25–6.15 mmol/g respectively, and nylon microparticles (50 mg) were activated with CDI alone over the concentration range of 1.54–15.4 mmol/g. All activation reactions were stirred at ambient temperature for time intervals up to a maximum of 30 min.

IgG from DAR serum was coupled to each of the activated microparticles (500 μg IgG/50 mg particles) in 4 ml of 0.5 M borate buffer pH 9.5 using end-over-end rotation for 30 min. After washing with ethanolamine/HCl, the particles were suspended in 0.05 M phosphate buffer pH 7.4 and stored at 4°C before assay. Each of the preparations was assessed as a solid-phase second antibody in an LH RIA using a rabbit primary antibody (see methods section).

Dynospheres required at least 30 mmol/g tresyl chloride to fully activate the microparticles whereas Dynospheres or nylon microparticles were fully activated with 3 mmol/g CDI. CDI therefore activates with greater efficiency.

Dynospheres, independent of activating agent, achieved maximal RIA binding after 10 min

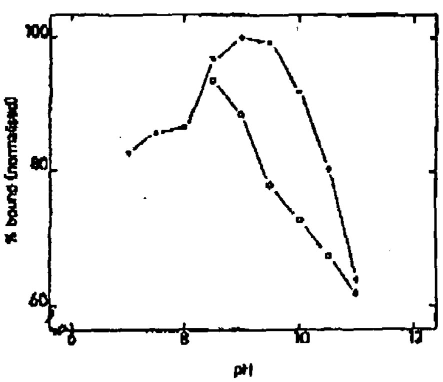

Fig. 1. Coupling efficiency as a function of pH for Dynospheres activated with tresyl chloride. (■——■): 0.5 M borate buffer (maximum binding 35%). (○——○): 0.5 M carbonate buffer (maximum binding 32%).

activation, whereas nylon microparticles required marginally longer, i.e., 15 min.

(2) Optimal conditions for covalent coupling

(a) Time of coupling

The rate of coupling of DAR IgG to each of the activated microparticles was examined over a 60 min period. In each case, independent of both types of microparticle and activation reagent, a 15 min reaction time gave a maximally efficient solid-phase second antibody preparation when examined in the test LH RIA.

(b) Coupling pH

Various buffers; acetate pH 3–5, phosphate pH 5–8, EPPS pH 7–9, borate pH 7–11 and carbonate pH 8.5–11, each at 0.5 M, were used to investigate the effect of pH on the coupling of DAR IgG to Dynospheres and nylon microparticles activated with either tresyl chloride or CDI. In each case the coupling time was 30 min.

Coupling to CDI-activated microparticles (Dynospheres or nylon) was independent of pH and buffer salt within the range pH 4–11. However, Dynospheres activated with tresyl chloride had a marked pH dependence (Fig. 1), pH 9.0–9.5 being optimal. Phosphate buffer, 0.1 M, pH 7.0 and borate buffer, 0.25 M, pH 9.0 were finally chosen for coupling to CDI activated nylon microparticles and Dynospheres respectively due to the lower non-specific binding levels achieved by each of the microparticles when used as solid-phase second antibodies in RIA.

(c) Effect of coupling buffer molarity

Coupling efficiency was also examined following changes in buffer molarity from 0.05 M to 0.5 M. A minimum of 0.25 M borate buffer, pH 9.0, was required for tresyl chloride activated Dynospheres, but coupling efficiency was independent of buffer molarity whenever CDI was the activating agent. 0.1 M phosphate buffer, pH 7.0, was chosen arbitrarily for coupling after CDI activation.

(d) Effects of changes in concentration of activated microparticles

Variation of activated microparticle concentrations from 5 to 40 mg/ml during the coupling of either CDI activated Dynospheres and nylon, or tresyl chloride activated Dynospheres to DAR IgG did not produce any change in coupling efficiency.

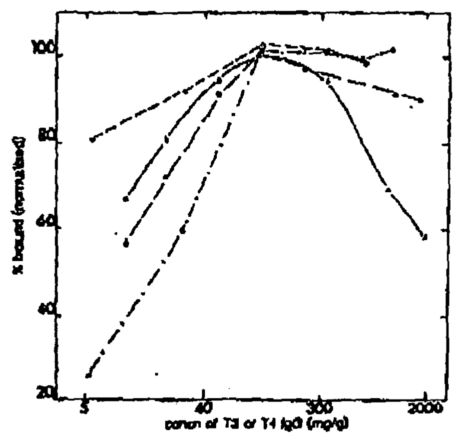

Fig. 2. Binding as a function of: (a) Concentration of sheep anti-T_3 IgG for CDI activated Dynospheres (●-·-●) or nylon (▲——▲) (maximum binding 65% and 77% respectively). (b) Concentration of sheep anti-T_4 IgG for CDI activated Dynospheres (O---O) or nylon (Δ---Δ) (maximum binding 47% and 85% respectively).

(e) Effect of IgG concentration

At this stage the more efficient reagent, CDI, was used to activate both Dynospheres and nylon microparticles to study the effect of IgG concentration on the coupling reaction. Several different IgG preparations were examined. DASG IgG and DAR IgG were coupled at concentrations ranging from 0.5 to 50 mg/g particles, whereas sheep anti-T_4 and sheep anti-T_3 were similarly coupled over a wider range of concentrations ranging from 5.0 to 2000 mg/g particles. Both solid-phase donkey antisera were examined for maximal binding attained following separation of the test LH RIA using either rabbit or sheep anti-LH primary antibody as appropriate. The solid-phase sheep antisera were used directly as primary antibodies in RIAs for T_4 or T_3 (Fig. 2). Maximal attainable binding in the respective RIAs was taken as indicative of the optimal IgG coupling concentration.

Not surprisingly, the concentration of IgG required for maximal binding in each case was dependent upon the respective antibody titres. DAR IgG was used at 10 mg IgG/g particles, DASG IgG at 20 mg/g particles and both sheep anti-T_4 IgG and sheep anti-T_3 IgG were used at 100 mg/g particles (Fig. 2) reflecting the low titres of the anti-thyroid antisera and the hyperimmune status of both precipitating sera.

(3) Summary of optimal activation and coupling conditions with CDI as activator

1 g of either microparticle in 20 ml acetone containing 0.5 g CDI was stirred vigorously for 15 min at room temperature. After washing in distilled water and recovery by filtration, the microparticles were resuspended in 50 ml 0.1 M phosphate buffer pH 7.0 with 20 mg of DASG or DAR IgG or 100 mg of sheep anti-T_3 or T_4 IgG. Coupling was achieved by rotation for 30 min at room temperature and residual active groups were neutralised by washing with a blocking agent (50 ml 1 M ethanolamine/HCl pH 9.5) for 60 min at room temperature. The microparticles were then resuspended in assay buffer at a concentration appropriate for immunoassay. On average, 1.5 mg IgG were coupled to 100 mg of nylon (as determined by the method of Schacterle and Pollack (1973)).

(4) Applications of solid-phase reagents to immunoassay

(a) Solid-phase second antibodies

(i) Time of incubation. DAR IgG and DASG IgG were covalently linked, under optimal conditions, to CDI-activated Dynospheres and nylon microparticles respectively. Both preparations, Dynospheres 0.5 mg/tube and nylon 1.0 mg/tube, were used to determine the minimum incubation period required to achieve maximal precipitation of the bound fraction in the test LH RIA using rabbit or sheep anti-LH primary antibody as appropriate. Nylon microparticles and Dynospheres were incubated at ambient temperature for time periods up to 60 min. Continuous shaking was unnecessary since both types of particle remained buoyant and immunoreactive throughout the 60 min incubation. Independent of the antibody coupled, a short incubation of 30 min was sufficient for maximal precipitation of the bound fraction.

(ii) Capacity. Since, in general, the efficiency of the solid-phase second antibody separation can be expected to deteriorate as the titre of the first antibody decreases, each of the optimally prepared reagents, DAR and DASG IgG covalently linked to both Dynospheres and nylon microparticles, was investigated to determine its effective operating range in terms of first antibody concentration. Non-immune IgG, rabbit or sheep, was added to either rabbit or sheep anti-LH serum as appropriate to simulate a range of first antibody titres decreasing from 1:250000 to 1:5000. Nylon microparticles (at 1 mg/tube), linked to either DAR or DASG IgG, retained their efficiency with first antibody titres as low as 1:5000. Dynospheres (at 0.5 mg/tube), however, linked to DASG and DAR IgG lost efficiency at titres of 1:25000 and 1:10000 respectively. This situation could be expected to improve if the particles were used at a concentration equivalent to nylon microparticles, i.e. 1 mg/tube.

(iii) Applications. Dynospheres or nylon microparticles linked optimally to either DAR or DASG IgG as appropriate have been applied successfully as solid-phase second antibodies in a wide range of immunoassays, including LH, FSH, GH and prolactin.

(b) Solid-phase primary antibodies

(i) Solid-phase RIA of thyroid hormones. Partially purified IgG from both sheep anti-T_4 and anti-T_3 sera coupled to Dynospheres and nylon microparticles under optimal conditions were examined for their utility in solid-phase RIA of serum thyroid hormones.

Antibody dilution curves, where the mass of solid-phase particles was reduced from 2000 to 30 μg/tube are illustrated in Fig. 3. Nylon microparticles gave binding in excess of 70% with both T_4 and T_3 antibodies at 2000 μg/tube. Antibodies linked to Dynospheres did not compare favourable suggesting a reduced IgG binding capacity. Binding to Dynospheres-linked anti-T_4 IgG was disproportionately low, 40% at 2000 μg/tube, indicating that the combined factors of poor solid-phase capacity and low antibody titre led to the

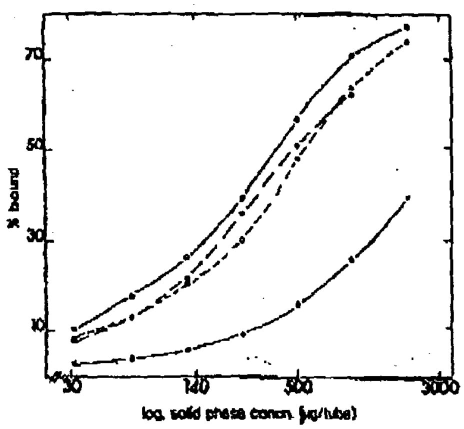

Fig. 3. Antibody dilution curves for solid-phase T_3 RIA (Dynospheres (●- - -●), nylon (O- - -O) and for solid-phase T_4 RIA (Dynospheres (●———●), nylon (O———O)). CDI activation in all cases.

production of a poor quality solid-phase inappropriate for use in RIA. However, if the Dynospheres were linked to a higher titre antibody, as occurred with the anti-T_3 IgG, this factor tends to compensate for the low capacity of the activated solid-phase providing a reagent suitable for use in RIA. In this case 50% binding can be achieved with 500 µg Dynospheres/tube.

Standard curves for the T_4 and T_3 RIAs with nylon anti-T_3, nylon anti-T_4 and Dynospheres anti-T_3 at 500 µg/tube and Dynospheres anti-T_4 at 1000 µg/tube were analysed to derive response-error relationships (RER) and intra-assay precision profiles for the respective solid-phase RIAs (calculated according to Ekins, 1983).

Analysis of the precision profiles derived for T_4 RIA indicated that both solid-phase antibody preparations (Dynospheres or nylon) gave similar assay performance. Both preparations gave assays with sensitivities of < 10 nmol/l, maximal precision (6% CV) within the clinically relevant dose range (50–150 nmol/l) and good precision (< 10% CV) maintained at 300 nmol/l. On balance, nylon was marginally superior.

A similar pattern was observed in T_3 RIA: Dynospheres exhibited better sensitivity than nylon, although both were adequate for clinical purposes. Both solid-phases gave maximal precision (4% CV) across the clinically relevant dose range (1.0–3.0 nmol/l) with nylon retaining better precision at high dose levels.

Discussion

Commercial Dynospheres and reprecipitated acid-hydrolysed nylon 6/6 microparticles possess many of the desirable features required of an ideal solid-phase matrix for immunoassay.

Dynospheres are uniform hydrophilic particles (about 3 µm in diameter) consisting of a polystyrene core surrounded by an acrylic shell containing hydroxymethyl methacrylate with a density of 1.07 g/cm³. These factors combine to provide a microparticle with surface groups (–OH) available for covalent linkage of antibodies and a capability of remaining buoyant during the majority of incubation periods encountered in immunoassay. Similarly, reprecipitated nylon microparticles (about 1 µm in diameter) remain buoyant and also have surface groups ($-NH_2$) available for covalent linkage.

A careful, critical, comparison of the two microparticles with respect to ease of activation with either tresyl chloride or CDI and application to immunoassay as either solid-phase first or second antibody reagents has been undertaken.

Analysis of the two activation procedures indicated optimal activation times of less than 15 min in each case, but optimal activation was achieved at a lower concentration for CDI (3 mmol/g) than tresyl chloride (30 mmol/g), confirming that CDI activates with greater efficiency. Coupling of antibodies to tresyl chloride activated microparticles was also markedly pH and molarity dependent being optimal at pH 9.0–9.5 in 0.25 M coupling buffer. Alternatively, antibody coupling to CDI-activated microparticles was pH and molarity independent over the ranges studied. These factors together with the high cost of tresyl chloride and the relatively hazardous nature of both tresyl chloride and pyridine led us to adopt CDI as the preferred activator. In general, the activation procedure outlined is highly practical for a busy clinical chemistry department since solid-phase reagents can be prepared rapidly within a single working day.

For solid-phase second antibody applications both types of microparticle were capable of remaining in suspension during the 60 min incubation periods studied. Both DAR and DASG linked to Dynospheres or nylon microparticles achieved maximal precipitation of the test-labelled antibody after 30 min incubation. This finding would allow a saving of 18–24 h compared with conventional liquid-phase second antibody systems. However, further analysis designed to simulate the effect of reducing the first antibody titre showed that the nylon microparticles had a greater capacity for coupling second antibody and consequently were able to maintain their separation effectiveness as the first antibody titre was reduced to 1:5000 dilution. Nevertheless, although nylon microparticles were more flexible in this respect, both particles behaved identically.

In conclusion, Dynospheres are of limited use where the titres of the antibodies are low since the costs involved in adding higher quantities of particles to compensate for this effect are not negligible. Nylon, whose costs are negligible since the particles are easily prepared from raw nylon 6/6 pellets, acts as a more flexible general solid-phase matrix. However, current work has indicated higher non-specific binding with nylon particles when used as a solid-phase primary antibody reagent, a factor which is presently being investigated together with the feasibility of using each of the microparticles in automated RIA systems.

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